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BRIEF COMMUNICATION

Novel *NLRP12* mutations associated with intestinal amyloidosis in a patient diagnosed with common variable immunodeficiency

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Abstract Heterozygous mutations in the *NLRP12* gene have been found in patients with systemic auto-inflammatory diseases. However, the NLRP12-associated periodic fever syndromes show a wide clinical spectrum, including patients without classical diagnostic symptoms. Here, we report on a 20-year-old female patient diagnosed with common variable immunodeficiency (CVID), who developed intestinal amyloidosis and carried novel compound heterozygous mutations in *NLRP12*, identified by whole exome and transcriptome sequencing. CVID is a primary immunodeficiency characterized by low serum immunoglobulins, recurrent bacterial infections and development of malignancy, but it also presents with a magnitude of autoimmune features. Because of the unspecific heterogeneous clinical features of the disease, a delay in diagnosis is common. Secondary, inflammatory (AA type) amyloidosis has infrequently been observed in CVID patients. Based on our case observation and a critical review of the literature, we suggest that *NLRP12* mutations might account for a small fraction of CVID patients with severe auto-inflammatory complications.

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1. Introduction

Common variable immunodeficiency (CVID) represents a heterogeneous subgroup of primary immunodeficiencies (PID), characterized by frequent and recurrent bacterial infections as a result of hypogammaglobulinemia and reduced specific antibody responses [1]. CVID is the clinically most prevalent PID, with an expected incidence of about 1 in 5000–50,000 [2]. Whereas most cases occur sporadically, up to 20% seem to be hereditary [3]. Although the underlying mechanism of the inborn humoral immunodeficiency is unknown in the majority of affected patients, genetic defects in several genes have been shown to be associated with the clinical CVID phenotype. Notably, most of these genes – the inducible T-cell co-stimulator (*ICOS*), tumor necrosis factor receptor superfamily member 13B (*TACI*), tumor necrosis factor receptor superfamily member 13C (*BAFFR*), *CD19* and *CD81*– are relevant for intrinsic B cell activation, proliferation and survival [4–8]. In contrast, there is a growing number of monogenetic conditions altering the global control of inflammatory processes, thereby causing CVID-like B cell deficiencies associated with severe autoimmunity. CVID patients with defects in the phospholipase Cγ_2 (*PLCG2*) and the protein C kinase δ (*PRKCD*) have been shown to present with a progressive decrease in $CD19^+$ B cells and lupus-like autoimmunity, cold-induced urticaria or inflammatory phenotypes reminiscent of systemic auto-inflammatory diseases (SAID) [9,10].

Here we investigated the molecular cause of a CVID patient with B cell lymphopenia, severely reduced immunoglobulin levels, and development of juvenile idiopathic arthritis (JIA) and intestinal inflammatory (AA) amyloidosis. Amyloidosis is characterized by protein deposition in the β-sheet structures of the extracellular matrix of tissues, leading to a progressive dysfunction of the related organs. Whereas primary amyloidosis (AL) exists together with idiopathic or plasma cell dyscrasias, secondary amyloidosis (AA) is more likely to develop when there is a chronic dysregulation of inflammatory processes [11]. In these patients, serum amyloid A is synthesized in the liver during unrestricted chronic inflammatory episodes, raising its plasma concentration up by about 1000 times [12]. AA-type amyloidosis has been described in CVID patients infrequently only, most likely because of the low awareness towards the condition, delayed treatment initiation for years and the tedious investigational procedures required to detect morphological changes [13–18].

Combined whole-exome and whole-transcriptome sequencing revealed two deleterious heterozygous mutations in *NLRP12* encoding the NALP12 NOD-like receptor, previously described in patients with hereditary periodic fever syndromes, as a potential molecular cause of this PID associated with severe autoimmunity in our index patient.

2. Patient and methods

2.1. Clinical and laboratory findings

The female index patient was the first-born offspring from consanguineous parents of Turkish descent with no family history of primary immunodeficiencies or auto-inflammatory diseases. Recurrent pulmonary infections and intermittent diarrhea led to several hospitalizations before the age of 8 years. At age 12, a diagnosis of common variable immunodeficiency was established based on severely decreased serum levels of IgG, IgA and IgM and the exclusion of secondary causes of hypogammaglobulinemia. The lymphocyte phenotype and vaccine responses were not investigated at that time, and specific vaccine responses were not assessed later on as those were not covered by health insurance at that time. A regular immunoglobulin substitution therapy was not initiated, and only single doses were applied before the age of 20 years. Henceforward, the patient was counseled at the rheumatology department because of polyarticular arthritis, in particular involving the shoulder, elbow and knee joint. Serological investigations including rheumatoid factor, ANA, p-ANCA, c-ANCA, and anti-dsDNA antibodies were all negative. A diagnosis of juvenile idiopathic arthritis was established and corticosteroids and low-dose methotrexate were administered. However, the refractory clinical course led to ankylosis in the knee joint and persistent movement constraints. At no time, skin rashes, urticaria, periodic fever or cold-induced symptoms were observed or reported.

At age 20, the patient was admitted to the hospital with complaints of abdominal pain and diarrhea lasting for 1 month. Physical examination showed a height of 150 cm (<3rd percentile), a weight of 48 kg (<3rd percentile) and pronounced splenomegaly and cervical lymphadenopathy. A chest computed tomography revealed condensed lymph node packages in the mediastinum, axillary, and supraclavicular areas, as well as post-pneumonic residues and bronchiectasis in the lower left lobe. These findings are in line with chest X-rays performed earlier, showing signs of lymphadenopathy and chronic inflammation. Pulmonary function was not investigated. Lung lymph node biopsies for histological examination revealed hyperplastic germinal centers with activated follicles of increased size (not shown). A PPD skin test and *Mycobacterium tuberculosis* and HIV PCR were negative. No pathogens were detected in the blood and fecal cultures. Her serum immunoglobulin levels showed a reduction of IgG (3.82 g/l), IgA (0.21 g/l) and IgM (0.16 g/l). Levels of IgE (17 kU/l), total protein (61 g/l) and serum albumin (38 g/l) were low-to-normal. CRP in serum was 29.2 mg/l and ESR 17 mm/h. Flowcytometric investigations revealed the following lymphocyte subset composition: $CD45^+CD3^+$ T cells of 79%, $CD45^+CD19^+$ B cells of 0.6%, and $CD3^-CD16/56^+$ NK cells of 5%. B cell subsets were not analyzed. The diagnosis of CVID was re-confirmed and the patient was started on intravenous immunoglobulins (IVIG) at 400 mg/kg every 3 weeks, which led to an increase in weight and height during the treatment period. In summary, the CVID phenotype was mostly characterized by viral pulmonary infections and bacterial pneumonias; no sinus infections were noted. However, the intestinal disease did not improve markedly.

Six months later, the patient presented again with severe chronic diarrhea. Flowcytometric tests indicated that the percentage of $CD45^+CD19^+$ B cells had declined to <0.1%, and B cell subset analysis could not be performed. As the substituted IgG levels reached 3.2 g/l only, and the total serum protein level was 41 g/l, intestinal or renal protein loss was suspected. Bed-side urine tests detected a considerable proteinuria; however, the protein loss within a quantitative 24-hour test was not at the nephrotic level (66 g/l). An enteropathy was suspected and several intestinal biopsies